Fig. 2

Step 1: Creation of unique Bsu36I restriction (CCTTAGG) site in 3' terminus of gamma-4 constant region:

Sequence encoding last 4 amino acids of human gamma-4 constant region:

TCT CTG GGT AAA [SEQ ID No.: 5]

Modified sequence encoding the same amino acids:

TCC TTA GGG AAG [SEQ ID No.: 6]

Step 2: PCR primers utilized for accomplishing such modification:

Primer 1:

5'γ4 oligo GGG ACC CAC GGG GTG CGA GGG C (Dra III) [SEQ ID No.: 7]

Primer 2:

3'γ4 oligo CTT CCC TAA GGA CAT GGA GAG GCT CTT CTG TGT GTG (Bsu36I) [SEQ ID No.: 8]

Primer 3:

5'γ1oligo GAT TCC TTA GGG AAG GCA GAG CCC AAA TCT AGT GAC (Bsu36I) [SEQ ID No.: 9]
ser

Primer 4:

3'γ1 oligo GCC GGA ATT CGG TAC GTG CCA AGC ATC CTC GTG C (EcoR I) [SEQ ID No.: 10]

Step 3: Three way ligation:

a. introduce new Bsu36I site at gamma 4 – hinge junction
b. add hinge and gamma 1 CH2 and CH3 domains
c. clone into DraIII-EcoRI sites of expression vector (VDJ-IgG4)